ESTABLISHMENT OF TISSUE CULTURE PLANTING MATERIALS OF GAC (*MOMORDICA COCHINCHINENSIS*) USING IMPROVED LIGHT EMITTING DIODES (LEDS) TECHNOLOGY AND SILVER NITRATE TREATMENT

CHEW HONG LIM

UNIVERSITI SAINS MALAYSIA

2021

ESTABLISHMENT OF TISSUE CULTURE PLANTING MATERIALS OF GAC (*MOMORDICA COCHINCHINENSIS*) USING IMPROVED LIGHT EMITTING DIODES (LEDS) TECHNOLOGY AND SILVER NITRATE TREATMENT

by

CHEW HONG LIM

Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

March 2021

ACKNOWLEDGEMENT

First and foremost, I would like to thank my parents and family for giving me the push and motivation for the whole duration of my research. Their unconditional support and encouragement keep me going through the obstacles and challenges encountered throughout my research journey.

I am forever indebted to my supervisor Professor Dr. Sreeramanan Subramaniam, for his extraordinary patience and his insights which assisted my research tremendously. His continuous presence regardless of time, enables invaluable consultations when required as well as moral support. His unwavering trust in me helps to instil confidence and sense of responsibility throughout my research. I would like to thank my co-supervisor Dr. Chew Bee Lynn for her advice and moral support in both my research. Her soothing demeanor helps to make the lab environment lively while maintaining the line between students and supervisor.

I am thankful to the Collaborative Research in Engineering, Science and Technology Centre (CREST) for awarding me with a scholarship to pursue my Master's degree which enabled the continuation and completion of my postgraduate studies. I am grateful to the School of Biological Sciences, Universiti Sains Malaysia, for providing the necessary facilities and dedicated staff required during the research.

Last but not least, I am thankful to my colleagues Yeow Lit Chow, Eyu Chan Hong, Dr Safiah binti Ahmad Mubbarak and every lab mate that provided me with constant companionship, experience, assistance and fruitful discussion.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ACRONYMS AND ABBREVIATIONS

$AgNO_3$	Silver Nitrate
ANOVA	Analysis of variance
CRD	Complete randomised design
DAMD	Directed amplification of minisatellite DNA
HCl	hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
ISSR	Inter simple sequence repeats
kb	Kilobase pairs
LED	Light Emitting Diode
mg/L	Milligram per litre
MS	Murashige and Skoog
NaOH	Sodium hydroxide
nm	Nanometre
PCR	Polymerase chain reaction
PGR	Plant growth regulator
SE	Standard error
SI	Similarity index
Tm	Melting temperature
v/v	Volume over volume

PENUBUHAN BAHAN TANAMAN KULTUR TISU GAC (MOMORDICA COCHINCHINENSIS) MENGGUNAKAN TEKNOLOGI DIOD PEMANCAR CAHAYA YANG DITAMBAH BAIK

ABSTRAK

Pokok gac (*Momordica cochinchinensis*) dari keluarga cucurbitaceae berasal dari wilayah Asia Tenggara. Buah ini telah digunakan secara tradisional sebagai makanan dan ubat-ubatan disebabkan tahap fitonutrien dan antioksidan yang tinggi terutamanya beta-karoten dan likopen. Eksplan nodal *M. cochinchinensis* telah digunakan untuk penubuhan sistem kultur tisu tumbuhan. Pengawalanturan pertumbuhan tanaman (PPT) terbaik untuk pertumbuhan semula tunas pada segmen eksplan nodal pokok gac yang telah diperolehi dengan menggunakan media separa pepejal MS yang mengandungi 4mg/L BAP dan telah menghasilkan 9.2 pucuk secara purata dan 2.247 sm panjang eksplan selepas 4 minggu. PPT yang optimum untuk induksi akar eksplan segmen nodal tanaman gac diperolehi dengan menggunakan media separa pepejal MS yang mengandungi 4mg/L IBA dan ini telah menghasilkan 13.2 akar secara purata dan 4.29 sm panjang eksplan selepas 4 minggu. Setelah memperolehi rawatan PPT yang optima untuk pertumbuhan, eksplan nodal gac diletakkan dibawah rawatan pelbagai jenis spektra diod pemancar cahaya (DPC) seperti putih (kawalan), hijau, merah jauh, merah, biru dan ungu (biru dan merah) selama 4 minggu. Spektra DPC merah menunjukkan kesan yang paling ketara adalah pada induksi tunas dan eksplan gac. Eksplan di bawah pencahayaan merah menghasilkan bilangan pucuk purata tertinggi iaitu 13.7 sm dan panjang purata 2.193 sm. Manakala jumlah akar tertinggi dihasilkan adalah 16.8 sm dengan panjang purata 1.897 sm. Untuk memantau

setiap perubahan atau perbezaan antara DPC berlainan dengan PPT yang telah dioptimakan, analisis histologi, biokimia dan molekular dijalankan pada anak pokok *M. cochinchinensis* dengan spektrum DPC. Kajian histologi menunjukkan bahawa pertumbuhan anak pokok di bawah pencahayaan DPC merah adalah yang terbaik berdasarkan pemerhatian di bawah mikroskop cahaya dan ini diikuti dengan spektra DPC ungu, merah-jauh dan hijau. Sementara itu, pertumbuhan anak pokok yang paling sedikit dipengaruhi adalah di bawah pencahayaan DPC biru. Analisis biokimia mendedahkan kandungan klorofil dari anak pokok yang telah dirawat bawah spektra DPC ungu adalah yang paling tinggi. Klorofil a, b, jumlah klorofil (a + b) dan kandungan karotenoid juga adalah tertinggi di bawah pencahayaan DPC ungu berbanding dengan lampu DPC lain. Aktiviti antioksidan dari anak pokok rawatan DPC masing-masing ditentukan berdasarkan ujian DPPH. Polimorfisme genetik telah ditentukan melalui analisis molekul DAMD dan ISSR. Penggunaan argentum nitrat dari kepekatan yang berbeza telah digunakan untuk induksi pokok gac hermafrodit melalui dalam keadaan *ex vitro* dan *in vitro*. Oleh itu, dapat disimpulkan bahawa kombinasi 4mg/L BAP dan 4 mg/L IBA dengan pencahayaan spektrum DPC merah adalah optima untuk pertumbuhan anak pokok gac secara *in vitro*. Kajian ini menunjukkan bahawa penerapan teknologi DPC adalah bermanfaat untuk propagasi *M. cochinchinensis* dan hasil penemuan ini dapat digunakan pada tumbuhan lain.

ESTABLISHMENT OF TISSUE CULTURE PLANTING MATERIALS OF GAC PLANT (*MOMORDICA COCHINCHINENSIS*) USING IMPROVED LIGHT EMITTING DIODES (LEDS) TECHNOLOGY

ABSTRACT

The Gac plant (*Momordica cochinchinensis*) of the cucurbitaceae family is native to the Southeast Asia region. The fruit has traditionally used as food and medicine due to their high levels of phytonutrients and antioxidants particularly beta-carotene and lycopene. *M. cochinchinensis* nodal explants used for the establishment of plant tissue culture system. The best plant growth regulator (PGR) for shoot regeneration in nodal segment explants of gac plant obtained by using semi-solid MS medium with 4mg/L BAP resulted an average of 9.2 shoots per explant and an average of 2.247 cm in length after 4 weeks. The best PGR for root induction for nodal segment explants of gac plant obtained by using semi-solid MS medium with 4mg/L IBA resulted an average of 13.2 roots per explant and 4.29cm in length after 4 weeks. After obtaining the optimum PGRs for the growth, gac nodal explants subjected with the treatment of various types of LED spectrums such as white (control), green, far-red, red, blue and purple [blue and red(1:1)] LEDs for duration of 4 weeks. Red LED spectrum displayed the most significant effect on shoot and root induction of gac explants. Explants under red illumination produced the highest average number of shoots which was 13.7 and an average of 2.193 cm in length. Meanwhile, the highest number of roots produced at 16.8 with an average of 1.897 cm in length. In order to monitor any changes or differences between various LED treatments with the optimised PGRs, histology, biochemical and molecular analyses were carried out on *M. cochinchinensis* plantlets of respective LED spectrum. Histological studies showed that plantlet growth under red LED illumination was the best based on the observations under light microscope followed by purple, far-red and green LED spectrum while the plantlet growth that least affected was by blue LED illumination. Biochemical analysis revealed the chlorophyll contents of plantlets of various LED spectrum where plantlets under purple LED conditions was enhanced, inducing the highest chlorophyll a, b, total chlorophyll (a + b) and carotenoid content amongst other LED lightings. Antioxidant activities of the plantlets of respective LED treatments were determined based on DPPH assay. Genetic polymorphism was determined using DAMD and ISSR molecular analysis. Utilisation of silver nitrate using various concentrations was used for the induction of hermaphrodite gac plant via *ex vitro* and *in vitro* conditions. Hence, it can conclude that 4mg/L BAP and IBA along with red LED spectrum were optimal for the growth of *in vitro* gac plant. This study demonstrated that the application of LED technology was advantages for the propagation of *M. cochinchinensis* and could applied to other plants.

CHAPTER 1

INTRODUCTION

Gac plant, a perennial melon scientifically known as *Momordica cochinchinensis* is a traditional medicinal plant which is indigenous and native to the tropical Asia regions such as Vietnam, Thailand, China, Laos and India (Chuyen et al., 2015; Leevutinun et al., 2015). This underutilised plant classified under the melon family known as *Cucurbitaceae* that includes bitter gourd, squash, cucumber and other related melons (Tinrat et al., 2014; Zheng et al., 2015). The bright-red gac fruits have known as giant spine gourd, red jackfruit, sweet gourd, Gac in Vietnam, Mu Bei Zi in China, Fak kao in Thailand and many other names in various Asian languages (Zheng et al., 2015).

Traditionally, gac fruits have used as food and medicine in the region abundant with it such as in Vietnam where the red fruit is an indigenous diet to the locals. The aril and ripen seeds of gac fruit used to prepare traditional Vietnamese cuisine known as xoi gac, a red glutinous rice dish, particularly during festive occasions such as weddings and new year celebration (Zheng et al., 2015). In Thailand, the fruit used in chilli paste and cooking curry (Wimontham & Rojanakorn, 2016). In India, the fruits harvested while small and green with immature seeds consumed as a vegetable (Kha et al., 2013). The spiny skin removed while the fruits are sliced and cooked occasionally with potato or bottle gourd. In some regions of India, the tender leaves and shoots of the plant are also consumed (Kha et al., 2013). Among all cucurbitaceous vegetables grown during the summer season, sweet gourd fetches a high price in the market (Sanwal et al., 2011).

Besides consumption, gac fruit used to aid in relieving of dry eyes as well as to promote healthy vision in Vietnamese folk medicine (Leevutinun et al., 2015). Moreover, the gac seeds utilised as Chinese traditional medicine in the treatment of liver and spleen disorders as well as sores (Kha et al., 2013). It also is known that the fruit contains a therapeutic value in the treatment of wounds, pus, haemorrhoids, swelling, diabetes and cancer in folk medicine (Burke et al., 2005; Tien et al., 2005).

Numerous studies have reported on the biological activities and health benefits of gac fruit extract, which narrows down to its antioxidant, antimicrobial and anti-cancer capabilities. Coined as ‘fruit from heaven" or "superfruit", various scientific studies highlighted that the gac fruit is rich in extraordinarily high levels of phytonutrients and antioxidants, particularly in beta-carotene and lycopene which both are very beneficial to human health (Burke et al., 2005). It observed that the antioxidant content of gac fruit is 5 to 10 folds higher compared to many lycopene-rich fruits and vegetable such as grapefruit and tomato (Kha et al., 2013). Besides carotenoids, gac fruit also provides an excellent source of Vitamin A and E, which included cosmetic components due to its high antioxidant efficacy. Tinrat et al. (2014) reported that ethanol extract of gac peel, pulp and aril exhibited good antimicrobial activities against six types of pathogenic bacteria strains, which includes both gram-positive and gram-negative bacteria.

On the other hand, Innun (2013) demonstrated the antimicrobial properties of gac extract with water and ethanol extract from the pulp or aril that showed pro-inhibition of growth of some bacteria tested. Clinical studies conducted by Leevutinun et al. (2015) confirmed that the cream product significantly reduces skin wrinkles, improves smoothness and moisture of skin when gac extract incorporated into the cosmetic cream. Formulated gac extract product is an effective anti-wrinkle cream.

The whole fruit itself is medicinally important based on numerous pharmaceutical analysis. Due to its high levels of carotenoids, the gac fruit exhibited exceptional high antioxidant properties and free radicals scavenging activities (Leevutinun et al., 2015). According to recent studies, several reports found that gac fruit extract possesses significant anti-cancer properties. As proclaimed by Yu et al. (2017), a phytochemicals investigation of gac fruit seed extract using ethanol was able to suppress cell proliferation of 4 human lung cancer cell lines. The main bioactive constituents identified were two major saponins, specifically gypsogenin and quillaic acid (Yu et al., 2017).

Moreover, Petchsak & Sripanidkulchai (2015) and Zheng et al. (2015) reported that the extract of gac seeds and aril found to have the capability to suppress breast cancer cell line via cell apoptosis. These observations have elevated the possibilities of anti-cancer therapeutic drug production from gac fruit. Similarly, Mazzio et al. (2015) reported that gac seed extract possesses the capability to induce neurite outgrowth based on high throughput screening of 1114 predominantly natural or herbal products. This remarkable result revealed that the gac fruit has a huge potential to be used for therapeutic application in central nervous system degenerative disease or injuries such as Alzheimer's and Parkinson's disease (Mazzio et al., 2015).

The potential health benefits of gac fruit consumption have attracted ample attention based on various scientific studies (Kubola & Siriamornpun, 2011). Furthermore, the increasing evidence of the potential of bioactive compounds in gac fruits to act as anti-cancer properties (Zheng et al., 2015) and other biological activities highlights the importance of developing gac fruit product as therapeutic drugs (Tinrat et al., 2014). Traditionally, cultivation of gac plants is through seed germination, stem cuttings and grafting. There were many problems associated with traditional plantation of gac plants such as seed dormancy that causes low multiplication rate and the dioecious condition of the gac plant (Sanwal et al., 2011). Moreover, the unpredictable sex ratio in gac seeds which often produced more male plants compared to female ones makes it difficult for cultivation and expansion in mass production of fruits as male plants do not bear fruits. The utility of male counterparts ends after supplying of pollen for fruit set on female plants (Sanwal et al., 2011). Additionally, gac fruit production is seasonal, making it less abundant compared to other fruits.

Light is the primary energy source for the growth and development of plants. LED lamps serve as a relevant artificial lighting source (Darko et al., 2014). LEDs influences photosynthesis, morphogenesis and physiological processes of a plant (Wang et al., 2016; He et al., 2017). LED commercial plant tissue culture laboratories are progressively utilising lights because of the advantages offered over conventional lighting systems (fluorescent light, halide metal and incandescent light bulbs) such as durability, wavelength specificity, compact size, cool emitting surface, lasting an operational lifetime and the ability to control spectral composition (Agarwal and Gupta 2016; Shukla et al., 2017; Batista et al., 2018). The usage of LED lights of different spectrum within the visible region from blue light (450–495 nm) to distant red (750–850 nm) attracted the attention of many research groups for the optimisation of plant growth, development and metabolism studies (Li et al., 2010; Lin et al., 2013; Golovatskaya and Karnachuk, 2015).

Tissue culture technology is a reliable and potent alternative in mass propagating plants under *in vitro* condition at a consistent and faster rate, yielding clones of parents from novel varieties while being able to bypass the sex ratio bottleneck (Sanwal et al., 2011; Wimalasiri, 2015). This aspect allows manipulation of media formulation and hormones (Bhatia, 2015) at controlled conditions with the aim to obtain large numbers of targeted gender of dioecious plant that can further be grown outside after acclimatisation.

In this study, the nodal segment of female gac vines chosen as the explants for the establishment of *in vitro* cultures. Using the nodal segment as the explant choice was assuring as the gender of the explant can be confirmed from the source. If seeds used, gender confirmation requires the plant to flower or to bud, which takes months. Seed germination produces more male plants compared to female ones which were difficult for cultivation. Four (4) types of PGR will be tested on the gac explants, where the best PGRs will utilise in the subsequent experiment under six types of LED conditions. LEDs can have peak emission wavelengths from ~ 250 nm (UV) to ~ 1000 nm (infrared) with more efficient performance and longevity well beyond any traditional lighting system (Rehman et al., 2017).

Biochemical, histological and DNA molecular analyses carried out on the induced gac plantlets under various spectrums of LED illuminations. Biochemical analyses such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Shen et al., 2010) and determination of total carotenoid and chlorophyll (Sarker et al., 2014) contents were carried out on gac plantlets to see if any changes occur under the different LED illuminations. Histological analysis carried out to detect plant cell changes amongst treatments and molecular analysis for any polymorphism or molecular abnormalities. This study aims to optimise the effect of PGRs and investigate the effects of different coloured LEDs on *M. cochinchinensis* explants on micropropagation system. An additional study carried out in this research on the possibility of the induction of hermaphrodite of gac plant via plant tissue culture system.

1.1 Research Objectives

This study carried out to suffice the following objectives:

i) To produce contamination free plantlets of *Momordica cochinchinensis* through surface sterilisation of *ex vitro* planting materials,

ii) To optimise regeneration of multiple shoots and root systems using various plant growth regulators with different planting materials under LED spectrum conditions,

iii) To determine the effects of different LED spectrum on *in vitro* propagated plant through histological, biochemical and DNA molecular analyses,

iv) To investigate the possibility of hermaphroditism induction on *Momordica cochinchinensis* via tissue culture technology.

CHAPTER 2

LITERATURE REVIEW

2.1 Cucurbitaceae

Cucurbitaceae known as cucurbits or the gourd family consists of 97 genera with 940–980 species (Schaefer & Renner, 2010). Cucurbitaceae comprises of monoecious, having both the male and female reproductive organs in the same plant species and dioecious, male and female reproductive organs on different plant species (Jeffrey, 1980). Its distribution is primarily tropical and subtropical, with few species reaching the temperate regions of the world. The aerial parts of all cucurbit species are sensitive to frost. Cucurbits are generally tendril-bearing monoecious or dioecious climbers. They are rarely without tendrils and are usually herbaceous annual vines or woody perennial lianas, exceptionally trees (Dendrosicyos). Cucurbits are often with rootstocks or tuberous roots and with leafless or succulent stems (Schaefer & Renner, 2010).

Cucurbits often attract nectary-tending ants with extra-floral nectaries on bracts, petioles, or flower buds (Agarwal & Rastogi, 2008; Schaefer & Renner, 2010). Other insects feed on other cucurbit plant parts such as flowers, leaves, and shoots. The Cucurbitaceae family stand among the highest of the plant families for number and percentage of species utilised for human consumption (Normah et al., 2013). The flowers are commonly greenish-white to yellow and are generally small (Jeffrey, 1980). The staminate, male flowers borne on an inflorescence that can be either racemose or cymose (Gerrath et al., 2008). The female flower or pistillate is generally solitary with an inferior ovary. The pollen of Cucurbitaceae family usually covered with a thick layer of oily yellow or orange coloured pollenkitt (Schaefer & Renner, 2010) and pollen-foraging bees are the predominant pollinators of Cucurbitaceae. The fruit is usually large, fleshy, with a hard outer covering and known as a pepo fruit type (Gerrath et al., 2008). Pepo fruit is a fleshy, several-seeded fruit that has developed from one flower of a single ovary divided into several carpels that develop to a firm or tough rind upon maturation. Members of the Cucurbitaceae family are generally grown in tropical and subtropical regions with limited representatives in colder temperate climates (Bharathi & John, 2013). A characteristic feature of cucurbits is the presence of cucurbitacins, toxic substances responsible for the bitterness that has erased from fruits through selective breeding but which are present in leaves and stems (Wimalasiri, 2015). Cucurbitacins are a group of bitter triterpenes confined mainly to the seeds of the cucurbit family (Chen et al., 2005). Biologically, they are useful as herbivore deterrents, although certain beetles adapted to these substances (Gillespie et al., 2003).

2.1.1 Gac fruit (*Momordica cochichinensis*)

Gac which belongs to the Cucurbitaceae and known as gourd or melon family is indigenous throughout Asia. The species name ‘‘*cochinchinensis*’’ derives from the northern part of Vietnam, the Cochinchina region, although the fruit is grown in many Asian countries (Vuong et al., 2006). It is grown in Thailand, Cambodia, Laos, Burma and other tropical areas and predominantly available in Bangladesh, China and India (Vuong et al., 2006).

The fruit is an underutilised perennial dioecious vegetable that been highly valued for its nutritional and medicinal qualities along with its wide range of uses. The whole fruit itself is medicinally important. Its fruit and seeds have been part of an indigenous diet, and the plant has been used as a traditional medicine throughout Southeast Asia from way back. In Vietnam, Gac is prized by natives for promoting longevity and vitality while commonly used as a colourant in traditional dishes and medicinal purposes. The bright red ripened seeds with a pleasant taste, cooked to impart its red colour, are traditionally used in Vietnamese cuisine in the dish 'Xoi Gac', a popular sticky rice dish made for weddings and New Year celebrations (Parks et al., 2013). The Chinese also use gac seeds in a traditional medicinal treatment known as “mubiezi” (Burke et al., 2005).

The seeds contained resolvent and cooling properties, utilised for liver and spleen disorders, wounds, haemorrhoids, swelling and pus (Shan et al., 2001). Gac fruit in Thailand is grown as a backyard vegetable or in small farms that have prominently processed for healthy foods and drinks. The young tips and fruit pulp are blanched first and served with chilli dressing or cooked in a Thai spicy mixed vegetable curry (Klungsupya et al., 2012). Its root used to cure hair fall, insect bite, cough, haemorrhoids, contraceptive in traditional medicine system and the elimination of poisonous substances (Nanthachit & Patoomratana, 2008). The maximal lycopene content in fresh aril of a fully ripe fruit reported being at 3.728 mg/g while β-carotene levels were 0.379 mg/g (Nhung et al., 2010). These levels are at least five (5) times higher compared to the lycopene content reported in tomatoes (Rao & Agarwal, 1999; Lenucci et al., 2009) and eight (8) times higher of the β-carotene content in carrots (Cefola et al., 2012).

Gac plant grows as dioecious vines, where the male and female flowers are on different plants while the vines can grow up to 20 m long (Parks et al., 2013). Rooted vine cuttings are more reliable than seedling production from seeds since germination by seed is proven to be difficult due to environmental factors such as seed dormancy. Flowering generally occurs between 2 to 3 months after planting and pollination is usually by hand pollination or occasionally insects. The fruit is typically round or oblong and varies about 13 cm in length and 10 cm in diameter. Fruit colour progressively changes from green to yellow, orange and finally red upon ripening (Do et al., 2019). The fruit is hard on harvest but rapidly turns soft, therefore leading to problems for transportation and shelf-life (Bharathi & John, 2013). It has spiny outer layer while its pulp is dense and light orange. The seeds are usually brownish or blackish and surrounded by red membranous sacs known as arils.

Gac fruits cultivated from Vietnam are typically round or ovoid, but one cultivar grown in India recorded to have an oblong shape (Osman, 2017). Dioecious condition, unavailability of improved varieties, seed dormancy, low multiplication rate and variable sex ratio in seed-based populations are significant bottlenecks in increasing the yield potential of this fruit species (Sanwal et al., 2011). Gac seeds reported to have trypsin inhibitors which cause dormancy (Huang et al., 1999) and might be affected by environmental conditions. Seed dormancy is not affected in *ex situ* conditions, but 80% of the seeds produced male plants (Parks et al., 2013).

2.1.2 Botany

The botanical description is useful in plant identification and the analysis of the phylogenetic relationships between species. The botanical description of *Momordica cochinchinensis* in India is reported by the literature (Bharathi & John, 2013) (Table 2.1). However, variations in the vegetative and reproductive characters exist based on the country along with the region the plant originates (Wimalasiri et al., 2016).

Table 2.1 Vegetative and reproductive morphological characters of *Momordica cochinchinensis*

Morphology	Parameter	Characteristics	Reference
Vegetative	Stem	Angular robust, glabrous	Bharathi & John, 2013
	Leaf shape and outline	Entire, broadly ovate or suborbicular outline	Bharathi & John, 2013
	Leaf lobes	3–5 palmately lobed	Bharathi & John, 2013
	Leaf size	10-16 cm	Bharathi & John, 2013
	Root	Tuberous	Bharathi & John, 2013
Reproductive	Male flowers		
	Bract	Sub apical, cucullate, reniform or suborbicular, 20–40 mm wide, rounded at base, scabrous, acute at apex, margins undulate, veins subparallel, very prominent outside	Bharathi & John, 2013; Wimalasiri et al., 2016
	Sepals	Coriaceous, 10–12, 4–8 mm, ovate-oblong/triangular, acute at apex, blackish, finely scabrid.	Bharathi & John, 2013; Wimalasiri et al., 2016
	Petals	Sub elliptic, conspicuously sub parallel-veined, 3 scales, at the base of the blotched petals, protecting the nectary; inner three petals with purple bull's eye mark at the base, short filaments,	Bharathi & John, 2013; Wimalasiri et al., 2016

	fleshy, 5–6 mm long, inserted at the base of the receptacle tube, anthers variable in size, ‘S’ shaped, and connective swollen.	
Female flowers		
Bract	Small or just like in males.	Bharathi & John, 2013; Wimalasiri et al., 2016
Sepals	Linear oblong, 4–10 mm long or just same as males	Bharathi & John, 2013; Wimalasiri et al., 2016
Petals	Same as the male flower.	Bharathi & John, 2013; Wimalasiri et al., 2016
Ovary	Ellipsoid oblong, 12–15 mm long, densely soft muricate.	Bharathi & John, 2013; Wimalasiri et al., 2016
Style	8–9 mm long.	Bharathi & John, 2013; Wimalasiri et al., 2016
Fruit		
Shape	Round or oval and shortly rostrate at the base.	Bharathi & John, 2013
Size	10–15 cm in length and 6–10 cm width.	Bharathi & John, 2013
Weight	350 g to 500 g or more.	Bharathi & John, 2013

Colour	Unripe fruits are green which turns orange/red upon ripening	Bharathi & John, 2013
Pericarp	Densely tuberculate with uniformly small round conical structures and yellow mesocarp.	Bharathi & John, 2013
Seeds		
Size	Vary, usually 1.5–2, 0.8–1.2 cm.	Bharathi & John, 2013; Wimalasiri et al., 2016
Shape	Broadly ovate penta-hexangular with flat sculptured surfaces and dentate margins.	Bharathi & John, 2013; Wimalasiri et al., 2016
Testa	Blackish Red aril (covering of the seeds)	Bharathi & John, 2013; Wimalasiri et al., 2016

Figure 2.1 Morphology of leaves, flowers (male, female), fruits (immature, mature) and seeds of *Momordica cochinchinensis*. (A) Leaves, **(B)** Male flower with broad reniform bracts, **(C)** Female flower with scabrous ovary, **(D)** Immature fruit, **(E)** Mature fruit, **(F)** Cross-section of the fruit and **(G)** Seeds with blackish testa. Scale bar = 2cm.

2.1.2 (a) Origin and distribution

The genus *Momordic*a originated from tropical Africa, and the Asian species were most likely the result of a long-distance dispersal event that occurred approximately 19 million years ago (Schaefer & Renner, 2010). The monoecious species have evolved from the dioecious species (Bharathi & John, 2013). The two monoecious species *Momordica charantia* (Bitter melon) and *Momordica balsamina L* (Balsam apple) formerly thought to have originated from Asia (Marr et al., 2004; Joseph & Antony, 2008). However, a study with mitochondrial and plastid DNA suggested they are of African origin (Schaefer & Renner, 2010).

Representatives of the genus *Momordica* distributed throughout Africa and Asia, but so far, only *Momordica charantia* is the widely cultivated crop (Bharathi & John, 2013). There are two botanical varieties of *Momordica charantia,* namely *Momordica charantia* var. *muricata* (syn. var. *abbreviata*) which is the wild type that can found in Africa and tropical Asia. The other variety which is *Momordica charantia* var. *charantia* is the cultivated variety (Walters & Decker-Walters, 1988). The dioecious species, *Momordica dioica* (spiny gourd), *Momordica cochinchinensis* (Gac) and *Momordica subangulata* subsp. *renigera* are under domestication interference (Bharathi & John, 2013). *Momordica cochinchinensis* hypothesised to have evolved in South Asia, most likely in the Cochinchina region of Vietnam and the evolution was independent of *Momordica dioica* (Mondal et al., 2006).

In contrast, morphological similarity and interspecific cross ability implied that *Momordica subangulata* ssp. Renigera (n = 56) originated from *Momordica dioica* (n = 28) and *Momordica cochinchinensis* (n = 28) as a result of a natural cross (Mondal et al., 2006; Bharathi et al., 2011). *Momordica balsamina L* and *Momordica sahyadrica* are found in the wild (Bharathi & John, 2013) where *Momordica balsamina L*. is distributed in western India, West Asia and Africa (Bharathi et al., 2011). *Momordica sahyadrica* is strictly restricted to India (Bharathi & John, 2013) and genetically diverse from other cucurbits.

2.2 Phytochemistry (β-carotene and lycopene)

Phytochemicals are chemicals derived from plants through primary or secondary metabolism, which are constituents that are essential for the survival and proper functioning of plants (Molyneux et al., 2007). Phytochemicals are produced as a defence mechanism against environmental stress conditions such as drought and extreme temperatures while playing a significant role in the adaptation of plants as well (Bourgaud et al., 2001). Some of the common phytochemicals reported in gac fruits are carotenoids, essential fatty acids, polyphenols and flavonoids (Kubola & Siriamornpun, 2011; Kha et al., 2013).

Momordica cochinchinensis is one of the most abundant sources of carotenoids in all known fruits and vegetables. The two predominant carotenoids in gac fruit are lycopene and β-carotene. It claimed that the lycopene concentration in a gac fruit is at least 5 times higher than other well-known fruits analysed (grapefruit, tomato, papaya, guava and watermelon) (Aoki et al., 2002; Rao & Rao, 2007). It is shown that gac aril has the highest known concentration of β-carotene of all fruits and vegetables (Vuong, 2000). The β-carotene content of gac is 8 times higher than the level in carrots, which recognised for the high levels in β-carotene content (Singh et al., 2001; Vuong et al., 2002; Kandlakunta et al., 2008).

The molecular structures of lycopene and β-carotene arranged in many forms which have conjugated double bonds in the chain that results in powerful antioxidant performance (Basuny, 2012). Other than the aril, the yellow pulp and peel are excellent sources of carotenoid and should not be overlooked as alternative carotenoid sources. Lutein has a higher concentration in the fruit skin or peel compared to the gac aril or pulp (Kubola & Siriamornpun, 2011). Countless studies have reported that lutein plays a vital role in age-related macular degradation (AMD) prevention (Greenberg et al., 2010; Ma & Lin, 2010). These components of Gac fruit usually discarded when the aril is scooped out and used for processing purposes. The aril constitutes only a minor proportion of the fruit weight, whereas the mesocarp, peel and seeds that make up the bulk of the fruit. Kha et al. (2013) reported that the proportion of aril in a gac fruit is less than 31% while the proportion of pulp, peel and seeds constitute about 90% of the total weight of the fruit. Fruit maturity was the most crucial factor, with the highest content of carotenoids in ripe fruits (Nhung et al., 2010).

2.2.1 α-Tocopherol (Vitamin E)

Vitamin E or α-tocopherol is an important fat-soluble antioxidative component in food and the human body that potentially plays a key role in preventing cardiovascular disease (Gaziano, 2004; Cordero et al., 2010), prevention of coronary heart disease (Rajasekhar et al., 2004; Ye & Song, 2008) and delaying Alzheimer’s disease (Kontush & Schekatolina, 2008; Nyam et al., 2009).

The concentration of vitamin E in a gac fruit which is 76 µg/ g of fresh weight, is significantly higher compared to other available fruits (Vuong et al., 2006). Vitamin E is a natural antioxidant that helps to protect the gac aril oil from oxidation (Vuong & King, 2003). In consumables, vitamin E could potentially preserve valuable phytonutrients available in Gac fruit from oxidation.

2.2.2 Polyphenolics and flavonoids

Polyphenolics are a diverse class of plant secondary metabolites. They are characterised structurally by the presence of one or more six-carbon aromatic rings as well as two or more phenolic hydroxyl groups. Polyphenolics appear to have many distinct functions in plants such as affecting the colour of leaves, flowers and fruit, anti-fungal function, anti-microbial, insect deterrence, chelation of toxic heavy metals, screening from damage by solar UV radiation and anti-oxidant protection from free radicals generated during the photosynthetic process (Gould et al., 2006).

Flavonoids are plant pigments that are synthesised from phenylalanine (Harborne, 1984) and generally display fabulous colours in the flowering parts of plants (Yao et al., 2004). Flavonoids comprise of a large group of polyphenolic compounds that are characterised by a benzo-y pyrone structure, which is available in vegetables and fruits. Besides their relevance in plants, flavonoids are important in human health because of their high pharmacological activities as radical scavengers.

Phenolic acids and flavonoids found in gac fruit potentially have beneficial effects on human health (Yao et al., 2004; Stevenson & Hurst, 2007; Abu Bakar et al., 2009). The phenolic acid content of *Momordica cochinchinensis* varies depending on the region of the fruit and its maturity stage (Kubola & Siriamornpun, 2011). The green mesocarp of *Momordica cochinchinensis* contained the highest levels of ferulic acid and p-hydroxybenzoic acid, which was 8 times higher than that detected in the green peel (Kubola & Siriamornpun, 2011). The total phenolic content of the immature fruit aril was twice more significant than the content of the peel (Kubola & Siriamornpun, 2011). However, the ripening of fruit reduced the total phenolic content in all parts of the fruit by more than 50% (Kubola & Siriamornpun, 2011).

The predominant flavonoids found in the aril are rutin and luteolin (Kubola & Siriamornpun, 2011). Myricetin found in all parts of the fruit, but the levels in the aril double the content found in the mesocarp (Kubola & Siriamornpun, 2011). The red aril contains a higher amount of rutin equivalent (RE) total flavonoid content (376 RE mg/g DW) compared to mature (302 RE mg/g DW) and immature mesocarp (285 RE mg/g DW) (Kubola & Siriamornpun, 2011). Furthermore, the catechin equivalent (CE) total flavonoid content of homogenised fruit was reported at 1.32 CE mg/g FW (Bharathi et al., 2014).

2.2.3 Fatty acids

The aril and seeds of Gac fruit are rich in fatty acids, mainly monounsaturated and polyunsaturated acids such as linoleic and oleic acids. Fresh and dried aril composes of fatty acids such as oleic, palmitic and linoleic acids. Seeds are rich in stearic, linoleic, palmitic and oleic fatty acids (Vuong et al., 2002; Ishida et al., 2004; Mai et al., 2013). Unlike the aril, the seeds usually discarded after consumption. Therefore utilisation of the seeds contributes to preventing waste disposal problems and maximising available resources. The large amounts of linoleic and oleic acids found in the aril may potentially contribute to human health by reducing LDL-cholesterol levels as well as having other antiatherogenic effects (Pariza, 2004).

The presence of high amounts of fatty acids and oil in the aril can also facilitate in the absorption and transportation of carotenoids into the human body (Vuong et al., 2002; Vuong & King, 2003). The total fatty acid content in Gac seeds is between 15.7% and 36.6% of the total weight of the seed (Ishida et al., 2004). The fatty acid composition includes stearic acid (54.5–71.7% by weight), linoleic acid (11.2–25.0%) and α-linolenic acid (0.5–0.6%). Several other types of fatty acids also found in gac seeds, but smaller amounts (Ishida et al., 2004). Gac aril oil contains high concentrations of oleic acid which is about 34 % of the total fatty acids. The aril oil can be utilised in addition to other prominent sources such as sunflower, palm and soya.

However, research on the effects of oleic acid in Gac fruit is still needed to affirm its benefits. Gac aril and seeds also contain α-linolenic acid that is beneficial to human health. Besides, α-linolenic acid has been regarded in some studies to play essential roles in reducing the incidence of cardiovascular diseases (DeFilippis et al., 2010; Rodriguez-leyva et al., 2010; Poudyal et al., 2011).

2.2.4 Pro-vitamin A activity

Vitamin A has many vital systemic functions in humans and can be produced within the body from specific carotenoids, notably β-carotene. B-carotene can be converted into Vitamin A in the body by the process called a pro-vitamin A precursor. The aril of *Momordica cochinchinensis* contains the highest concentration of β-carotene, over eight (8) times higher than the β-carotene content reported in carrots (Cefola et al., 2012).

A clinical trial on Vietnamese children found that a 30-day supplement of fresh gac fruit aril increased the plasma β-carotene and vitamin A concentration compared to the control group (steamed rice) and a group given synthetic β-carotene, indicating the bio-availability of β-carotene in *Momordica cochinchinensis* aril (Vuong et al., 2002). This bio-availability of β-carotene in *Momordica cochinchinensis* aril might be due to the presence of a higher trace of monounsaturated and polyunsaturated acids in the aril compared to synthetic β-carotene supplement, which has reported to promote the intestinal absorption of β-carotene in humans (Castenmiller & West, 1998; Yeum & Russell, 2002; Maiani et al., 2009). The study by Vuong et al. (2002) suggested that dietary intake of *Momordica cochinchinensis* aril has the potential to treat vitamin A deficiency that is especially noticeable in third world countries.

2.3 Antioxidant activity

An antioxidant is a compound present in the diet which can lower the formation of reactive oxygen species (ROS), such as pro-oxidants and free radicals that produced during aerobic cell respiration (Pulido et al., 2000). Fruit extracts of gac have shown anti-oxidative activities precisely due to their carotenoid and phenolic compounds (Kubola & Siriamornpun, 2011).

In a study on the various parts of the fruit, the aril of the mature fruits had the highest antioxidant activity while the seed extract had the lowest activity (Kubola & Siriamornpun, 2011). Aside from the ripe fruit, homogenised extract of immature green gac fruit also showed antioxidant activity of 0.058 mg/g, and 0.45 mg/g equivalent to the ascorbic acid determined using the ferric ion reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assays, respectively (Bharathi et al., 2014).

The antioxidant activity of *Momordica cochinchinensis* is affected by moisture content, the drying and processing temperature. An aril powder, prepared with 10% maltodextrin by spray drying at 120°C with a moisture content of 4.9% shown to have an antioxidant activity equivalent to 1.4 mmol Trolox per gram in the 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay (Kha, 2010) but the antioxidant activity reduced when the moisture content increased to about 6% (Kha et al., 2011). The processing temperature of 80°C caused isomerisation of 16% of the lycopene from the all-trans to the cis isoform which resulted in a 1.5 times higher antioxidant activity than at 50°C (Phan-Thi & Waché, 2014). However, the differences in antioxidant properties between lycopene isomers have not clearly explained in the literature.

2.4 Eco-geographical influences

The country and region of the origin of fruit and vegetable reflect climatic and geographical differences, including rainfall, temperature, sun exposure, elevation and other factors. These differences have corresponding to the variation in carotenoid content of fruits such as tomatoes (Aherne et al., 2009), mangoes (Mercadante & Rodriguez-Amaya, 1998), cashew apples (Assunção & Mercadante, 2003) and apricots (Munzuroglu et al., 2003; Dragovic et al., 2007). Variation in the carotenoid content (Table 2.2) might be attributed to differences in collection sites of *Momordica cochinchinensis*, but no systematic analysis has conducted.

The carotenoid content is known to be affected by the temperature during fruit development and an optimum temperature for this has been identified for tomatoes. The optimum temperature for lycopene synthesis ranges between 12 to 32°C for tomatoes (Dumas et al., 2003) but similar information not available for gac fruit. Exposure to such a wide range of temperatures influences the production of secondary plant metabolites, including carotenoids (Rice-Evans et al., 1996). Direct sunlight and excessive solar radiation are the leading causes in the reduction of the bioaccumulation of carotenoids in tomatoes (Helyes et al., 2007; Pék et al., 2011). Gac fruit found in tropical and temperate climates. However, the effects of temperatures have not been studied for carotenoid accumulation yet, and this will be important when considering growth sites for future agricultural production.

Fruits grown under field conditions produce higher lycopene concentrations compared to hydroponic farms and could be due to multiple factors including the exposure under the sun, cultivation practices, fertiliser usage and water usage (Kuti & Konuru, 2005). Field-grown cherry tomatoes had 1.6 times higher lycopene content compared to those grown under hydroponic conditions (Kimura & Rodriguez-Amaya, 2003; Kuti & Konuru, 2005). However, these conditions have not studied for their effects on the carotenoid accumulation in *M. cochinchinensis*.